P. .NT COOPERATION TREA.

From the INTERNATIONAL BUREAU

PCT

To:

BIRD, William, E.
Bird Goën & Co
Vilvoordsebaan 92
B-3020 Winksele
BELGIQUE

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

Date of mailing (day/month/year)	
30 octobre 2001 (30.10.01)	
Applicant's or agent's file reference K1291-PCT	**IMPORTANT NOTIFICATION**
International application No. PCT/EP00/03765	**International filing date** (day/month/year) 26 avril 2000 (26.04.00)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
PLUYMERS, Wim Rega Institute for Medical Research Minderbroedersstraat 10B B-3000 Leuven Belgium	BE	BE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
PLUYMERS, Wim Naamsesteenweg 282 B-3001 Heverlee Belgium	BE	BE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Idhir BRITEL
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 16 November 2000 (16.11.00)	
International application No. PCT/EP00/03765	**Applicant's or agent's file reference** K1291-PCT
International filing date (day/month/year) 26 April 2000 (26.04.00)	**Priority date** (day/month/year) 26 April 1999 (26.04.99)
Applicant DEBYSER, Zeger et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

24 October 2000 (24.10.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer S. Mafla
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

14

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
K1291-PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/03765	26/04/2000	26/04/1999
International Patent Classification (IPC) or national classification and IPC C12N15/86		
Applicant K.U. LEUVEN RESEARCH & DEVELOPMENT et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
24/10/2000	13.09.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Wimmer, G Telephone No. +49 89 2399 7347

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/03765

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-29 as originally filed

Claims, No.:

1-61 with telefax of 09/08/2001

Drawings, sheets:

1/4-4/4 as originally filed

Sequence listing part of the description, pages:

1-4 (SEQ ID NOs. 1-2), as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 27.

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 27 are so unclear that no meaningful opinion could be formed (*specify*):
   see separate sheet

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☒ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-26, 28-61
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-26, 28-61
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-26, 28-61
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

Re Item III

Non-establishment of opinion.

Subject-matter of claim 27 is defined in a product-by-process manner. Consequently, it cannot be determined if substances already known in the art will fall under the terms of this claim. Claim 27 was therefore not examined.

Re Item IV

Lack of unity of invention.

1) Based on original claims 1-29, the application was found to be lacking unity of invention.

   I) **original claims 1-3**:
   A detection method for intracellular integrase activity using a promoterless reporter gene;

   II) **original claims 4-12 completely and claims 17-29 partially**:
   Packaging construct for a lentiviral or complex retroviral vector based on a synthetic gag or pol gene; a synthetic retroviral gag or pol gene or a region of a retroviral gag or pol protein in a eucaryotic cell, the expressed retroviral protein being expressed at a level to provide detectable activity of the wildtype function of the expressed retroviral protein in the eucaryotic cell; a eucaryotic expression vector comprising said gene or a region thereof; a method of transfecting a eucaryotic cell using said expression vector; a eucaryotic cell line harboring said synthetic gene or a region thereof; a transgenic non-human animal harboring said synthetic genes or a region thereof;

   III) **original claims 13-16 completely and claims 17-29 partially**:
   A method for preparing a synthetic gene encoding a retroviral protein or part of such protein which is enzymatically active in a target eucaryotic cell.

No International Search Report had been established for the third group defined above. In reply to the Invitation to Restrict or to Pay Additional Fees, the applicant elected to pay one additional examination fee; consequently, groups I and II as defined above were subject to this preliminary examination.

2) The applicants are informed that amended claims 1-61 again appear to contain three independent inventions as follows:

I) **Invention 1** claims 1-26:
A detection method for intracellular integrase activity using a promoterless reporter gene;

II) **Invention 2** claims 28-38:
Packaging construct for a lentiviral or complex retroviral vector based on a synthetic gag or pol gene, and uses thereof;

III) **Invention 3** claims 39-61:
A synthetic retroviral gene enzymatically active in an eucaryotic cell, wherein non-preferred codons within the retroviral gene were replaced with codons preferred in mammalian cells, and wherein the GC content of said retroviral gene was adapted to between 53 and 63%.

These groups are not so linked to form a single general inventive concept, and therefore represent three different inventions.
However, since examination fees were paid for groups I and II as defined in section IV.1, and as a service of the EPO, no further invitation to Restrict or to Pay Additional Fees is extended at this moment.

Re Item V

Reasoned statement under Art. 35(2) PCT with regard to novelty, inventive st p or industrial applicability.

1) Reference is made to the following documents (the document numbering corresponds to their order of citation in the international search report):

D3: US-A-5 811 270 (GRANDGENETT DUANE P) 22 September 1998 (1998-09- 22) cited in the application

D4: US-A-5 434 065 (MAHAN MICHAEL J ET AL) 18 July 1995 (1995-07-18)

D6: WO 98 12207 A (GEN HOSPITAL CORP) 26 March 1998 (1998-03-26) cited in the application

D7: HOLLER T P ET AL: 'HIV1 INTEGRASE EXPRESSED IN ESCHERICHIA COLI FROM A SYNTHETIC GENE' GENE,NL,ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, vol. 136, 22 December 1993 (1993-12-22), pages 323-328, XP000199775 ISSN: 0378-1119

D10: WO 98 34640 A (DAVIES MARY ELLEN M ;PERRY HELEN C (US); FREED DANIEL C (US); LIU) 13 August 1998 (1998-08-13)

Invention 1

Novelty under Art. 33(2) PCT.

2) Claim 1 refers to a detection method for intracellular integrase activity, using a promoterless reporter gene.
Such a method is not described in the prior art. Document D3 describes a method for assessment of integrase activity, wherein integrase activity occurs in vitro, and which depends on the presence of a unique restriction site.
Document D4 describes an assay which is similar to that of the application, in that it uses a promoterless reporter gene, which is expressed from a promoter upstream of site of integration of the donor DNA molecule. However, this method uses homologous recombination for integration of the donor DNA, and can not be used for assessing integrase activity.

Claims 1 - 26 are therefore novel.

Inventive Step under Art. 33(3) PCT.

3) With regard to subject-matter of claim 1, document D4 can be viewed to be the closest prior art. The problem solved by the application, was therefore a further application of the method of D4. In the opinion of the IPEA, the solution proposed by the present invention, the assessment of integrase activity, is not obvious from the prior art, and furthermore requires modifications to the method of D4 (such as the introduction of flanking retroviral LTRs).
An inventive step is therefore acknowledged for the methods of claims 1 - 26.

Invention 2

Novelty under Art. 33(2) PCT.

4) The prior art describes modified retroviral sequences, which have been adapted to preferred codon usage in eucaryotic cells (e.g. D10 discloses accordingly modified HIV gag coding sequences). However, these modified retroviral sequences have not been used in the creation of packaging vectors, or according cell lines.
Subject-matter of claims 28-38 is therefore novel.

Inventive Step under Art. 33(3) PCT.

5) Document D10 discloses HIV gag sequences with modified codon usage, although for the creation of HIV vaccines. In the opinion of the IPEA, the prior art does not directly lead the skilled person to use the gene of D10 for the creation of a packaging construct. An inventive step is therefore formally acknowledged for subject-matter of claims 28-38.

Inv ntion 3

Novelty under Art. 33(2) PCT.

6) Document D6 describes the modification of several genes, wherein the codon usage is adapted to the preferred use in mammalian cells. Specifically, D6 shows the modification and successful high-level expression of HIV gp120. D6 describes that this method can be generally applied to a wide variety of genes (see pg. 45/23 - 46/4), and specifically mentions the preferred extension of this method to other HIV genes, such as the pol gene (pg. 3; claim 14).

The entities and methods of present invention III (claims 39-61) differ from the prior art insofar as codon usage is biased to obtain a GC content of between 53 and 63%.

The prior art does not state a preferred GC content of genes modified for preferred codon usage. Therefore, in the absence of evidence that the modification of the gag gene as disclosed in D10, or the modification of the pol gene proposed in D6, would automatically lead to a gene with GC content of between 53-63%, subject-matter of claims 39-61 is considered to be novel.

Inventive Step under Art. 33(3) PCT.

7) The modified genes and according methods of invention III differ from the prior art (e.g. D6) insofar as that they are limited to retroviral genes which exert an enzymatic function, and which have a GC content of between 53 and 63%.

While the extension of e.g. the method of D6 to enzymatically active retroviral genes is obvious and even proposed in D6, none of the prior art documents discloses a preferred GC content.
Moreover, since the average GC content in mammalian genes is approx. 40% (Lewin, B. (1994) Genes V, Oxford University Press, N.Y., 111), the entities and methods of the present invention appear to be limited to a selected range of modified retroviral enzymatic proteins, which is not obvious in the light of the prior art.

The involvement of an inventive step is therefore acknowledged for subject-matter of claims 39-61.

Re Item VI

Certain published documents (Rule 70.10 PCT).

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO 00/39302	06.07.2000	30.12.1999	31.12.1998

Claims

1. A detection method for intracellular integrase activity using a promoterless reporter gene.

2. The method according to claim 1, wherein the integrase activity is present in cell culture.

3. The method according to claim 1 or 2, wherein the integrase activity is present after transfection of an integrase gene.

4. The method according to any of claims 1 to 3, where the integrase activity is performed by an integrase protein.

5. The method according to claim 4, wherein the integrase protein is a wild type integrase enzyme.

6. The method according to any previous claim, wherein the integrase protein is mutated.

7. The method according to any of claims 1 to 6, wherein the integrase gene is mutated in order to obtain an optimised codon usage.

8. The method according to any previous claim, wherein the integrase gene is a synthetic gene having a portion of the wildtype codons relaced by other codons.

9. The method according to any previous claim, wherein the integrase protein is retroviral.

10. The method according to any of claims 1 to 9, where the integrase protein is lentiviral.

11. The method according to claim 10, wherein the integrase protein is an HIV integrase.

12. The method according to any previous claim, wherein the reporter gene is one of a

lucif rase, GFP and an antibiotic selection marker.

13. The method according to any of the claims 1 to 11, wherein the reporter gene is a cytotoxic drug resistance gene.

14. The method according to any previous claim, wherein a reporter gene construct is generated from the reporter gene and the construct is used as the substrate of an enzymatically active retroviral protein expressed from a synthetic retroviral pol or gag gene, the synthetic gene having modified codon usage compared with a wildtype gene, the synthetic gene being for expression of a retroviral pol or gag gene or a region of a retroviral pol or gag gene in a eukaryotic cell, the expressed retroviral protein being at a level to provide detectable activity of a wild type function of the expressed retroviral protein in the eukaryotic cell.

15. The method according to claim 14, wherein the synthetic gene is for the expression of a lentiviral pol or gag gene or a region of a lentiviral pol or gag gene in a eukaryotic cell, the expressed lentiviral protein being at a level to provide detectable activity of a wild type function of the expressed lentiviral protein in the eukaryotic cell.

16. The method according to claim 14 or 15, wherein the synthetic gene is for the expression of a retroviral or lentiviral gag or pole gene or a region of a retroviral or lentiviral gag or pol gene where the gene or region thereof, after codon optimization for a eukaryotic host in which it is expressed, contains a GC nucleotide pair content between 53 and 63 %, more preferably between 55 and 61 %, and the expressed gene is expressed at a level to provide detectable enzymatic activity of the expressed retroviral or lentiviral protein in the eukaryotic cell.

17. The method according to any of claims 14 to 16, wherein the expression of the gag or pol gene or the region thereof is independent of retroviral regulatory proteins.

18. The method according to any of claims 14 to 17 wherein the retroviral protein is a lentiviral gag or pol protein or a fragment thereof.

19. The method according to claim 18 wherein the retroviral protein is an HIV gag or pol protein or a fragment th reof.

20. The method according to any of claims 14 to 19, wherein the detectable activity of the enzymatic function includes at least promotion or stimulation of the integration of DNA fragments into the host cell DNA, preferably the chromosome if the host cell.

21. The method according to any of the claims 14 to 20, wherein the eukaryotc cell for expression of genes is a mammalian cell.

22. The method according to any of the claims 14 to 21 wherein the expressed protein has an expression level of at least 200 % compared to the expressed wild type gene in a eukaryotic cell.

23. The method according to any of the claims 14 to 21 containing a synthetic gene comprising the sequence of Fig 2A or homologs thereof which have a GC content between 53 and 63 % preferably between 55 and 61 percent.

24. The method according to any of claims 1 to 23, wherein a reporter gene construct is generated from the reporter gene and the construct contains an internal IRES.

25. The method according to any previous claim, wherein the reporter gene codes for an enzyme.

26. Use of a method according any of the claims 1 to 25 for screening for integrase inhibitors.

27. An integrase inhibitor obtained by the method of claim 26.

28. Packaging construct for a lentiviral or complex retroviral vector based on a synthetic retroviral pol or gag gene, the synthetic gene having modified codon usage compared with a wildtype gene, the synthetic gene being for expression of a retroviral pol or gag gene or a region of a retroviral pol or gag gene in a eukaryotic cell, the expressed retroviral protein being at a level to provide detectable activity of a wild type function of the expressed retroviral protein in the eukaryotic cell.

29. Packaging construct according to claim 28 wherein the synthetic gene is for the expression of a l ntiviral pol or gag gene or a region of a lentiviral pol or gag gene in

a eukaryotic cell, the expressed lentiviral protein being at a level to provide detectable activity of a wild type function of the expressed lentiviral protein in the eukaryotic cell.

30. Packaging construct according to claim 28 or 29, for the expression of a retroviral gag or pole gene or a region of a retroviral gag or pol gene where the gene or region thereof, after codon optimization for a eukaryotic host in which it is expressed, contains a GC nucleotide pair content between 53 and 63 %, more preferably between 55 and 61 %, and the expressed gene is expressed at a level to provide detectable enzymatic activity of the expressed retroviral protein in the eukaryotic cell.

31. Packaging construct according to any of claims 28 to 30, wherein the expression of the gag or pol gene or the region thereof is independent of retroviral regulatory proteins.

32. Packaging construct according to any of claims 28 to 31, wherein the retroviral protein is a lentiviral gag or pol protein or a fragment thereof.

33. Packaging construct according to claim 32 wherein the retroviral protein is an HIV gag or pol protein or a fragment thereof.

34. Packaging construct according to any of claims 28 to 33, wherein the detectable activity of the enzymatic function includes at least promotion or stimulation of the integration of DNA fragments into the host cell DNA, preferably the chromosome if the host cell.

35. Packaging construct according to any of the claims 28 to 34, wherein the retroviral protein is a protease, reverse transcriptase, integrase or a polyprotein gag-pol precursor thereof.

36. Packaging construct according to any of the claims 28 to 35, wherein the eukaryotic cell for expression of genes is a mammalian cell.

37. Packaging construct according to any of the claims 28 to 36, wherein the expressed protein has an expression level of at least 200 % compared to the expressed wild

type gene in a eukaryotic cell.

38. Packaging construct according to any of the claims 28 to 37 containing a synthetic gene comprising the sequence of Fig 2A or homologs thereof which have a GC content between 53 and 63 % preferably between 55 and 61 percent.

39. A method of transfecting a eukaryotic cell using the expression vector in accordance with any of claims 59 to 61.

40. A eukaryotic cell line harboring the synthetic gene or region of a gene in accordance with any of the claims 50 to 58.

41. The eukaryotic cell line according to claim 40, wherein the retroviral enzymatically active protein is expressed using a constitutive, inducable or tissue specific promoter.

42. The eukaryotic cell line according to claim 40 or 41, wherein the expression is stable.

43. A transgenic animal harboring the synthetic gene or part of a gene in accordance with any of the claims 50 to 58.

44. The transgenic animal according to claim 43, wherein the expression of the synthetic gene or part of a gene is induced by an inducable promoter or by a tissue-specific promoter.

45. The transgenic animal according to claim 43 or 44, comprising a mammal.

46. A method for preparing a synthetic gene or part of a gene encoding a retroviral protein or part of such a protein which is enzymatically active in a target eukaryotic cell, comprising the steps of:

1) identifying a group of genes from the total set of genes of the target eukaryotic cell which encode proteins which are naturally expressed easily

and/or in high concentrations in the target cell;

2) determining the codon sequences of these identified genes and from these sequences a preferred codon usage and a preferred nucleotide pair frequency;

3) using the preferred codon usage, identify the non-preferred codons in the natural gene encoding the enzymatically active protein;

4) replacing one or more of the non-preferred codons with one or more preferred codons encoding the same amino acids as the replaced codons while biasing the replacement to obtain the preferred nucleotide pair frequency, the preferred nucleotide pair frequency being a GC nucleotide pair content of between 53 and 63%, more preferably between 55 and 61%.

47. The method according to claim 46, wherein the replacement step is carried out based on a random choice between alternative codons encoding the same amino acid at each position using a random number generator and biasing the choice of alternative codons based on the preferred codon usage to obtain the preferred nucleotide frequency.

48. A method for gene transfer in a eukaryotic cell expressing the synthetic gene or region of the gene in accordance with any of the claims 50 to 58.

49. A method according to claim 48, wherein the synthetic gene is transiently expressed or is stably integrated in said cell.

50. A synthetic retroviral *gag* or *pol* gene or a region of a retroviral *gag* or *pol* gene for the expression of a retroviral *gag* or *pol* protein in a eukaryotic cell, the retroviral gene having non-preferred codons when referred to the eukaryotic cell, the number of non-preferred codons being such that replacement of all the non-preferred codons by preferred codons for the eukaryotic cell results in a GC nucleotide pair content of 65% or higher, the synthetic gene having a GC nucleotide pair content of between 53 and 63%, more preferably between 55 and 61% and the expressed retroviral protein is expressed at a level to provide det ctable enzymatic activity of th expressed retorviral protein in the eukaryotic cell.

51. The synthetic gene according to claim 50, wherein the expression of the *gag* or *pol* gene proteins is independent of retroviral regulatory proteins.

52. The synthetic gene according to claim 50 or 51, wherein the retroviral protein is a lentiviral *gag* or *pol* protein.

53. The synthetic gene according to claim 52, wherein the lentiviral protein is an HIV *gag* or *pol* protein.

54. The synthetic gene according to any of claims 50 to 53, wherein the detectable activity of the enzymatic function includes at least promotion or stimulation of integration of DNA fragments into the host cell DNA, preferably the chromosome of the host cell.

55. The synthetic gene according to claim 54, wherein the retroviral protein is a protease, a reverse transcriptase, an integrase protein or a polyportein gag-pol precursor thereof.

56. The synthetic gene according to any of the claims 50 to 55, wherein the eukaryotic cell is a mammalian cell.

57. The synthetic gene according to any of the claims 50 to 56, wherein the expression of the protein is at a level at least 200% of that expressed by the wild type gene in the eukaryotic cell.

58. The synthetic gene according to any of the claims 50 to 57 comprising the sequence of Fig. 2A or homologs thereof which have a GC content between 53 and 63%, preferably between 55 and 61%.

59. A eukaryotic expression vector comprising the synthetic gene or region of a gene in accordance with any of the claims 50 to 58.

60. The expression vector according to claim 59, further comprising a constitutive or an inducible or a tissue-specific promoter.

61. The expression vector according to claim 59 or 60, comprising a plasmid, a mammalian or an insect virus.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
K1291-PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/03765	26/04/2000	26/04/1999
Applicant		
K.U. LEUVEN RESEARCH & DEVELOPMENT et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 7 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[X] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [X] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[ ] the text is approved as submitted by the applicant.

[X] the text has been established by this Authority to read as follows:

SYNTHETIC GENE FOR EXPRESSING ACTIVE RETROVIRAL PROTEIN IN EUKARYOTES

5. With regard to the **abstract,**

[ ] the text is approved as submitted by the applicant.

[X] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 4

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[ ] None of the figures.

Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

The present invention features a synthetic gene or region of a gene which has an amended codon usage compared with the wild-type gene and which is for the high level expression of a retroviral protein in eukaryotic cells, the expressed retroviral protein having enzymatic activity in the eukaryotic cell. In addition, the invention features a synthetic gene or region of a gene encoding a retroviral enzyme or part of a retroviral enzyme normally expressed in a mammalian or other eukaryotic cell wherein at least one non-preferred codon in the wild-type gene encoding the enzyme has been replaced by a preferred codon encoding the same amino acid. The retroviral protein may be a protease, reverse transcriptase, integrase protein or a polyprotein gag-pol precursor thereof. In one embodiment the retroviral protein with enzymatic activity is a lentiviral protein. In other embodiments the enzymatically active protein is a i(pol) enzyme. In more preferred embodiments, the enzymatically active protein is a lentiviral integrase In an even more preferred embodiment the enzyme is an HIV enzyme. In more preferred embodiments the enzymatically active protein is HIV integrase. The present invention also includes a detection method for intracellular integrase using a promoterless reporter gene.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/03765

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 3
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 3

Present claim 3 relates to a detection method using as the substrate a product defined in claims 17 to 26. However, present claim 17 (and dependent ones) relates to products defined by reference to a desirable characteristic, namely being expressed at a level to provide detectable activity of the wild-type function of the expressed retroviral protein in the eukaryotic cell.

The claim covers all products having this characteristic, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT).

An attempt is made to define the product by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search could only be carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the product wherein , as described in claim 18 and more specifically in the description page 13 line 11 to 14 line 1, a synthetic HIV-1 integrase gene wherein the GC code content would be increased up to 59%.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/03765

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/86 C12N5/10 C12N7/04 C12N15/49 C07K14/16
A61K48/00 C12N15/00 A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, STRAND, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BUSHMAN, F. D. ET AL.: "Retroviral DNA integration directed by HIV integration protein in vitro" SCIENCE, vol. 249, 28 September 1990 (1990-09-28), pages 1555-1558, XP000953086 LANCASTER, PA US page 1556, column 3, paragraphs 2,3 --- -/--	1,2

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 January 2001	08. 03. 2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chambonnet, F

3

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	KATZ, R.A. ET AL.: "The avian retroviral IN protein is both necessary and sufficient for integrative recombination in vitro" CELL, vol. 63, 5 October 1990 (1990-10-05), pages 87-95, XP000953087 CELL PRESS, CAMBRIDGE, NA., US ISSN: 0092-8674 page 88, column 1, line 1 - line 6 page 93 ---	1,2
X	US 5 811 270 A (GRANDGENETT DUANE P) 22 September 1998 (1998-09-22) cited in the application column 1, line 45 -column 2, line 10; claims 1,3 ---	1-3
A	US 5 434 065 A (MAHAN MICHAEL J ET AL) 18 July 1995 (1995-07-18) the whole document ---	1,2
A	US 5 468 629 A (CALHOUN CORNELIA) 21 November 1995 (1995-11-21) the whole document ---	1,2
A	WO 98 12207 A (GEN HOSPITAL CORP) 26 March 1998 (1998-03-26) cited in the application	3
X	page 1, line 16 -page 6, line 3; example 1; table 1 page 18, line 1 -page 21, line 4; table 2 page 26, line 11 - line 23; claims 1-14,25-28 ---	4,5
A	HOLLER T P ET AL: "HIV1 INTEGRASE EXPRESSED IN ESCHERICHIA COLI FROM A SYNTHETIC GENE" GENE,NL,ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, vol. 136, 22 December 1993 (1993-12-22), pages 323-328, XP000199775 ISSN: 0378-1119 page 326, column 2, paragraph D ---	1-3
P,X	CHEREPANOV P, SURRATT D, TOELEN J, PLUYMERS W, GRIFFITH J, DE CLERCQ E, DEBYSER Z.: "Activity of recombinant HIV-1 integrase on mini-HIV DNA." NUCLEIC ACIDS RES. 1999 MAY 15;27(10):2202-10., 15 May 1999 (1999-05-15), XP000877353 the whole document ---	1-3

-/--

3

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 36481 A (CORBEAU PIERRE ;KRAUS GUNTER (US); UNIV CALIFORNIA (US); WONG STAA) 9 October 1997 (1997-10-09) the whole document	1
X	WO 98 34640 A (DAVIES MARY ELLEN M ;PERRY HELEN C (US); FREED DANIEL C (US); LIU) 13 August 1998 (1998-08-13) the whole document	4,5
Y	HAAS J ET AL: "CODON USAGE LIMITATION IN THE EXPRESSION OF HIV-1 ENVELOPE GLYCOPROTEIN" CURRENT BIOLOGY,GB,CURRENT SCIENCE,, vol. 6, no. 3, 1 March 1996 (1996-03-01), pages 315-324, XP000619599 ISSN: 0960-9822 page 315, column 2, paragraph 2	4,5
X	SCHNEIDER R ET AL: "Inactivation of the human immunodeficiency virus type 1 inhibitory elements allows rev-independent expression of gag and gag/protease and particle formation" JOURNAL OF VIROLOGY,THE AMERICAN SOCIETY FOR MICROBIOLOGY,US, vol. 71, no. 7, July 1997 (1997-07), pages 4892-4903, XP002137891 ISSN: 0022-538X	4
Y	the whole document page 10, last paragraph page 5, paragraph 4 - last paragraph	4,5
P,X	ZUR MEGEDE J, CHEN MC, DOE B, SCHAEFER M, GREER CE, SELBY M, OTTEN GR, BARNETT SW: "Increased expression and immunogenicity of sequence-modified human immunodeficiency virus type 1 gag gene." J VIROL 2000 MAR;74(6):2628-35, XP002157414 page 2, paragraph 3 -page 3, paragraph 2 page 5, last paragraph; figure 1 page 10, last paragraph	4,5
T	KOTSOPOULOU E. ET AL.: "A REV-INDEPENDENT HUMAN IMMUNODEFICINCY VIRUS TYPE 1 (HIV-1)-BASED VECTOR THAT EXPLOITS A CODON OPTIMIZED HIV-1 GAG-POL GENE" JOURNAL OF VIROLOGY., vol. 74, no. 10, May 2000 (2000-05), pages 4839-4852, XP002152133 ICAN SOCIETY FOR MICROBIOLOGY US the whole document	4,5

-/--

3

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
E	WO 00 39302 A (CHIRON CORP) 6 July 2000 (2000-07-06) page 3, line 15 -page 5, line 10; figures 5,7 page 21, line 30 - line 34; claims 1-11,39,40,53-58; examples 2.1,,2.2.1. -----	4,5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/03765

Box I Observations wh re certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 3
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 3

Present claim 3 relates to a detection method using as the substrate a product defined in claims 17 to 26. However, present claim 17 (and dependent ones) relates to products defined by reference to a desirable characteristic, namely being expressed at a level to provide detectable activity of the wild-type function of the expressed retroviral protein in the eukaryotic cell.

The claim covers all products having this characteristic, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT).

An attempt is made to define the product by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search could only be carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the product wherein , as described in claim 18 and more specifically in the description page 13 line 11 to 14 line 1, a synthetic HIV-1 integrase gene wherein the GC code content would be increased up to 59%.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/03765

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5811270 A	22-09-1998	NONE	
US 5434065 A	18-07-1995	US 5512452 A	30-04-1996
		US 5571688 A	05-11-1996
US 5468629 A	21-11-1995	NONE	
WO 9812207 A	26-03-1998	US 6114148 A	05-09-2000
		AU 4355697 A	14-04-1998
		CN 1237977 A	08-12-1999
		CZ 9900968 A	15-09-1999
		EP 0929564 A	21-07-1999
		HU 9904239 A	28-04-2000
		PL 332431 A	13-09-1999
WO 9736481 A	09-10-1997	AU 2721997 A	22-10-1997
		CA 2249349 A	09-10-1997
		EP 0903981 A	31-03-1999
WO 9834640 A	13-08-1998	AU 6271198 A	26-08-1998
		CN 1252075 T	03-05-2000
		EP 0969862 A	12-01-2000
		HU 0001347 A	28-08-2000
		NO 993810 A	07-10-1999
		PL 335050 A	27-03-2000
		SK 106699 A	12-06-2000
WO 0039302 A	06-07-2000	AU 2221600 A	31-07-2000
		AU 2487300 A	31-07-2000
		AU 2596600 A	31-07-2000
		WO 0039303 A	06-07-2000
		WO 0039304 A	06-07-2000

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/86, 5/10, 7/04, 15/49, C07K 14/16, A61K 48/00, C12N 15/00, A01K 67/027

A2

(11) International Publication Number: WO 00/65076

(43) International Publication Date: 2 November 2000 (02.11.00)

(21) International Application Number: PCT/EP00/03765

(22) International Filing Date: 26 April 2000 (26.04.00)

(30) Priority Data:

99201306.0	26 April 1999 (26.04.99)	EP
00200171.7	18 January 2000 (18.01.00)	EP

(71) Applicant *(for all designated States except US)*: K.U. LEUVEN RESEARCH & DEVELOPMENT [BE/BE]; Groot-Begijnhof, Benedenstraat 59, B-3000 Leuven (BE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: DEBYSER, Zeger [BE/BE]; Rega Institute for Medical Research, Minderbroedersstraat 10B, B-3000 Leuven (BE). DE CLERCQ, Erik [BE/BE]; Rega Institute for Medical Research, Minderbroedersstraat 10B, B-3000 Leuven (BE). CHEREPANOV, Peter [RU/BE]; Rega Institute for Medical Research, Minderbroedersstraat 10B, B-3000 Leuven (BE). PLUYMERS, Wim [BE/BE]; Rega Institute for Medical Research, Minderbroedersstraat 10B, B-3000 Leuven (BE).

(74) Agents: BIRD, William, E. et al.; Bird Goën & Co, Vilvoordsebaan 92, B-3020 Winksele (BE).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

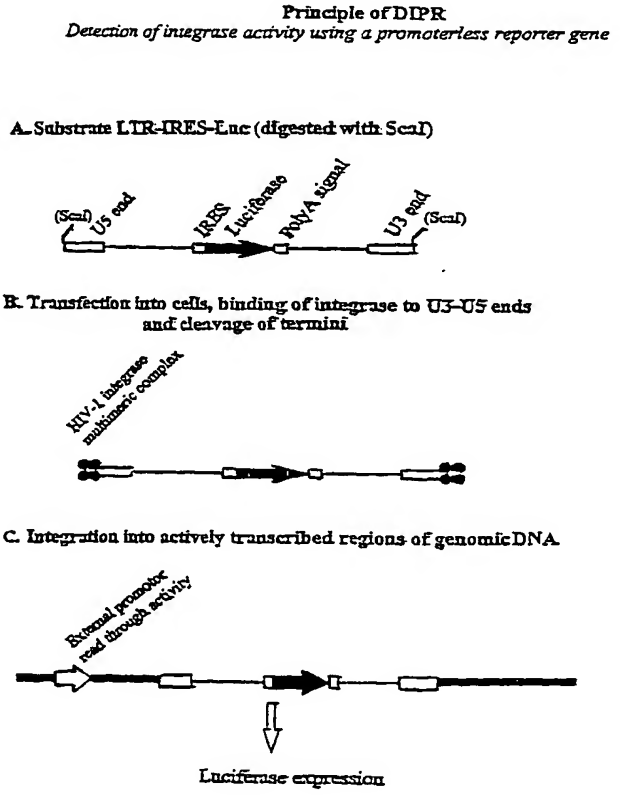
Published

Without international search report and to be republished upon receipt of that report.

(54) Title: SYNTHETIC GENE FOR EXPRESSING ACTIVE RETROVIRAL PROTEIN IN EUKARYOTES

(57) Abstract

The present invention features a synthetic gene or region of a gene which has an amended codon usage compared with the wild-type gene and which is for the high level expression of a retroviral protein in eukaryotic cells, the expressed retroviral protein having enzymatic activity in the eukaryotic cell. In addition, the invention features a synthetic gene or region of a gene encoding a retroviral enzyme or part of a retroviral enzyme normally expressed in a mammalian or other eukaryotic cell wherein at least one non-preferred codon in the wild-type gene encoding the enzyme has been replaced by a preferred codon encoding the same amino acid. The retroviral protein may be a protease, reverse transcriptase, integrase protein or a polyprotein gag-pol precursor thereof. In one embodiment the retroviral protein with enzymatic activity is a lentiviral protein. In other embodiments the enzymatically active protein is a *pol* enzyme. In more preferred embodiments, the enzymatically active protein is a lentiviral integrase. In an even more preferred embodiment the enzyme is an HIV enzyme. In more preferred embodiments the enzymatically active protein is HIV integrase. The present invention also includes a detection method for intracellular integrase using a promoterless reporter gene.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
2 November 2000 (02.11.2000)

PCT

(10) International Publication Number
WO 00/65076 A3

(51) International Patent Classification[7]: C12N 15/86, 5/10, 7/04, 15/49, C07K 14/16, A61K 48/00, C12N 15/00, A01K 67/027

(21) International Application Number: PCT/EP00/03765

(22) International Filing Date: 26 April 2000 (26.04.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
99201306.0 26 April 1999 (26.04.1999) EP
00200171.7 18 January 2000 (18.01.2000) EP

(71) Applicant *(for all designated States except US)*: **K.U. LEUVEN RESEARCH & DEVELOPMENT** [BE/BE]; Groot-Begijnhof, Benedenstraat 59, B-3000 Leuven (BE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **DEBYSER, Zeger** [BE/BE]; Korbeek-losestraat 108, B-3001 Heverlee (BE). **DE CLERCQ, Erik** [BE/BE]; Parklaan 9, B-3360 Lovenjoel (BE). **CHEREPANOV, Peter** [BE/BE]; Brusselsestraat 128, B-3000 Leuven (BE). **PLUYMERS, Wim** [BE/BE]; Naamsesteenweg 282, B-3001 Heverlee (BE).

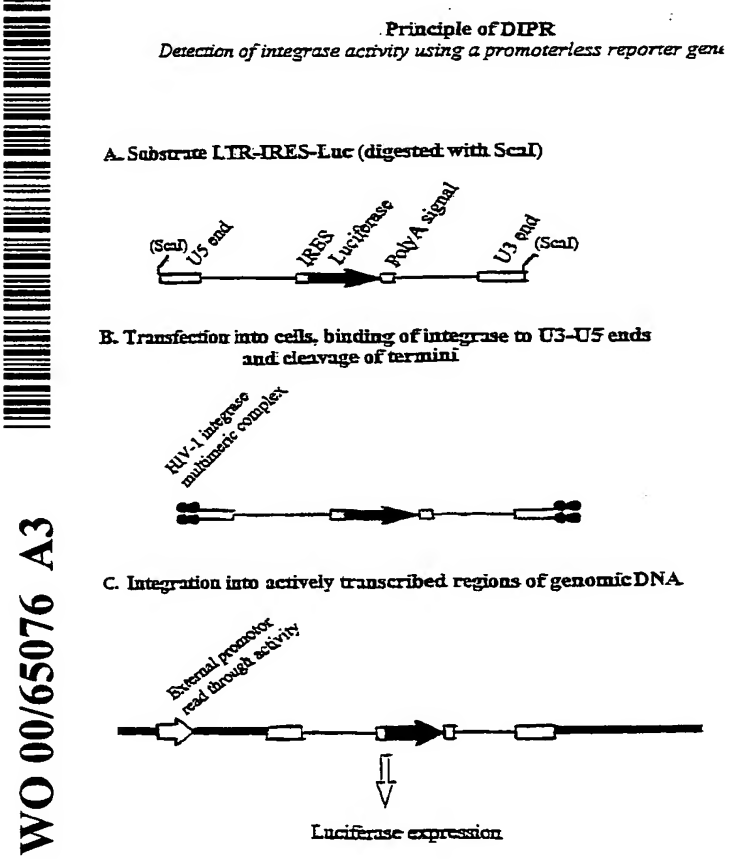
(74) Agents: **BIRD, William, E.** et al.; Bird Goën & Co, Vilvoordsebaan 92, B-3020 Winksele (BE).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent

[Continued on next page]

(54) Title: SYNTHETIC GENE FOR EXPRESSING ACTIVE RETROVIRAL PROTEIN IN EUKARYOTES



(57) Abstract: The present invention features a synthetic gene or region of a gene which has an amended codon usage compared with the wild-type gene and which is for the high level expression of a retroviral protein in eukaryotic cells, the expressed retroviral protein having enzymatic activity in the eukaryotic cell. In addition, the invention features a synthetic gene or region of a gene encoding a retroviral enzyme or part of a retroviral enzyme normally expressed in a mammalian or other eukaryotic cell wherein at least one non-preferred codon in the wild-type gene encoding the enzyme has been replaced by a preferred codon encoding the same amino acid. The retroviral protein may be a protease, reverse transcriptase, integrase protein or a polyprotein gag-pol precursor thereof. In one embodiment the retroviral protein with enzymatic activity is a lentiviral protein. In other embodiments the enzymatically active protein is a *pol* enzyme. In more preferred embodiments, the enzymatically active protein is a lentiviral integrase. In an even more preferred embodiment the enzyme is an HIV enzyme. In more preferred embodiments the enzymatically active protein is HIV integrase. The present invention also includes a detection method for intracellular integrase using a promoterless reporter gene.

WO 00/65076 A3

(AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
13 December 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.